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INFLUENCES OF MICROBIAL POPULATIONS
ON AQUATIC NUTRIENT CYCLES AND SOME
ENGINEERING ASPECTS

Leonard J. Guarraia, et al

Environmental Protection Agency
Washington, D. C.

May 1972

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Influences of Microbial Populations on
Aquatic Nutrient Cycles and Some Engineering Aspects

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Technical Studies Report

TS - 00 - 72 - 06

U.S. Environmental Protection Agency
Office of Water Programs
Water Quality and Non-Point Source Control Division
Water Quality Protection Branch
Fresh Water Pollution Control Section
Washington, D.C. 20460

May 1972

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Acknowledgements

The authors wish to thank our secretary Mrs. Nyla Linthicum who has labored tirelessly and with patience and good humor in the preparation of this report and who suffered excessive eye strain in the translation of the written word to the final typed word.

We appreciate the reviews and helpful comments of K.M. Mackenthun and L.E. Keup.

Cover Photograph

Courtesy Mr. Curtis Ross - U.S. Environmental Protection Agency - From Project Hypo, an intensive study of the Lake Erie Central Basin Hypolimnion and Related Surface Water Phenomena.

Front Cover

Picture of a "black patch" where the sulfide layer has penetrated up to the surface of the sediment.

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EPPATA SHEET

The following corrections are to be incorporated into the Influences Of Microbial Populations On Aquatic Nutrient Cycles And Some Engineering Aspects:

- P. 25 line 7 should read "...particles in the sediments at the..."
P. 30 line 13 should read "... Y_a = initial bacterial..."
P. 32 line 20, new paragraph should begin with "The increase in..."
P. 35 last line should read "...are processes expressing gains or losses in phytoplankton mass."

The following definitions should be included after Table 2:

Y_{co} = colored water lakes

Y_{cl} = clear water lakes

SD = Secchi Disc (m-l)

COND = specific conductance ($\mu mho/cm$)

TON = total organic nitrogen (mg N/l)

TP = total phosphorus (mg P/l)

PP = primary production (mg C/m³/hr)

CHA = chlorophyll a (mg/m³)

CR = cation ratio $\left(\frac{Na + K}{Mg + Ca} \right)$

Table 3

Should read F ratio

Figures 5 and 6

TSI = Trophic State Index

r = correlation coefficient

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SUMMARY

Domestic and industrial wastes, chemical fertilizer runoff from farms, suburban lawns and golf courses, heavy metals, and silt from land development all contribute to the eutrophication process. The addition of such agents to the aquatic ecosystem can result in an algal bloom. However, the cause and effect relationships between many changes in water quality and the causative flora and fauna may not be clearly recognizable as due to bacterial metabolism.

Proliferation of endogenous microbial populations caused by eutrophication leads to the establishment of anaerobic environments through increased respiration or biochemical oxygen demand (BOD). One of the causes of excessive eutrophication in water is the release of carbon dioxide, organic carbon compounds and the mineralization of nutrient elements which are the end-products of microbial catabolism. As a consequence of an increased rate of eutrophication, lakes age prematurely, rivers may become choked with algae or aquatic plants and benthic fauna may become smothered.

Further distortion of biological productivity is brought about by the ability of the bacteria to concentrate heavy metals such as mercury. It is the bacterial population that serves as a base for the food chain. Zooplankton further enhance metal concentration by feeding on bacteria. The plankton are in turn utilized by such organisms as shrimp, mollusks, and small fish as a food source. The consequence of this contamination in the food chain is that commercially important organisms are either killed or are rendered unfit for human consumption.

Establishment of species of bacteria new to an area can be important. Alterations in microbial flora can be caused by the discharge of human or industrial sewage into waterways. The introduction of pathogenic flora renders an area unsuitable for human use. An alteration of the nutrient cycles can also cause a change in the fauna.

Engineering control of natural systems in various stages of eutrophy must consider microbial responses to system changes. Limitation of one nutrient without limiting others may cause unexpected environmental responses. Development for predicting system response thus far includes deterministic ecological models which consider bacteria, phytoplankton and zooplankton populations based on nutrient concentrations and statistical models which predict eutrophic conditions based on watershed development or waste inputs. Using these tools various control schemes can be tested.

Clearly, all bacterial metabolic activities are not detrimental to the environment. Bacteria are involved in the cycling of phosphorus, nitrogen, carbon, sulfur, hydrogen, and other elements thus providing a nutritional base for the food chain. Degradation of complex organic molecules such as the hydrocarbons, detergents, phenolics, and alcohols is brought about by bacterial metabolism. Natural organic compounds such as cellulose, starch, and chitin are also degraded in the normal catabolic process of bacteria. All of these organic molecules ultimately are degraded to carbon dioxide and water for use by green plants. These catabolic activities are part of the normal life process in the environment and allow for the cycling of nutrients for reuse in the food chain.

CONCLUSIONS

Microbial influence on water eutrophication is generally accepted as significant. Aquatic microbial populations have been shown to be either the basic agents or among the primary effectors in the cycling of phosphorus, sulfur, nitrogen and carbon. There is a general qualitative understanding in nutrient cycling; however, many quantitative cycling rates, as governed by microbial populations, are unknown. With respect to microbially mediated nutrient cycling, the following conclusions can be made:

1. In the phosphorus cycle the involvement of microbes has been demonstrated. However, the extent and mechanisms of the involvement, the effects of physical and chemical perturbations caused by man's activities, and the implications to the total biological system are not well defined.
2. The transition of inorganic sulfur in the aquatic environment is regulated by two metabolically distinct groups of bacteria: the sulfate reducers and sulfate oxidizers. Hydrogen sulfide of biological origin is exclusively the result of the sulfate reducers. Conversely, sulfuric acid of biological origin is the result of sulfur oxidizing bacteria. These two metabolic groups also are implicated in the nitrogen, phosphorus and carbon cycles. Excessive organic pollution which depletes available oxygen supplies apparently will enrich for the sulfate reducing bacteria resulting in high sulfide concentrations. However, the direct relationships between various types of

3.

pollution to sulfur availability, sulfide or sulfate production and environmental dystrophy is not well defined.

3. Fixation of elemental nitrogen is the result of a select group of free living microbes; however, the transition of nitrogen through the various valence states is mediated by a large number of microbes. The influence of various pollutants on the nitrogen fixers and the possible consequences are not understood.
4. Microbial involvement in the carbon cycle is complex and involves not only carbon dioxide fixation but the degradation and synthesis of innumerable complex organic compounds. Subsequently, microbial biomass serves as a food source for higher organisms. When microbial activity is incapable of degrading organic material a dystrophy results. Peat bogs and accumulation of non-biodegradable synthetic organic compounds are examples of results from an imbalance in bacterial degradation. As added stresses are placed on the aquatic ecosystem, the consequences to the natural biological synthetic and degradative processes are often not predictable.
5. The effects of many types of pollutants on the microorganisms involved in the nitrogen, sulfur, phosphorus and carbon cycles are not well known. An understanding of the relationships of these cycles with each other and their function within the total ecosystem is needed. Such an understanding would permit more more efficient and effective waste management practices and the ability to

anticipate and perhaps prevent pollution disasters.

6. Whether certain pollutants selectively stimulate or inhibit the growth of specific microbial species is unknown. The ecological consequences of species selection processes and how to deal with these possibilities are not known. Species selection may be manifested in a variety of ways; for example, death of fish may be caused by growth of fish pathogens, excessive sulfide pollution, or establishment of an anaerobic environment.
7. The consequences to the aquatic environment when the indigenous microbial flora is altered are unknown. For example, shellfish feed on certain microflora; alteration of the foodbase can cause shellfish to die or become unfit for human consumption. The specific microbial flora can be changed by introduction of agricultural, domestic and industrial pollution into the water.
8. Since there are a variety of pollutants, organisms other than fecal coliform and fecal streptococci bacteria may be introduced and need to be recognized as indicators of pollution. This is important both from a health as well as economic point of view.
9. Engineering control of lake eutrophication requires the development of mathematical models which includes phytoplankton "production function" which accurately predict the ecosystem response to watershed changes.

Ultimately, the inclusion of political and economic factors must be incorporated in such models to produce the most publicly acceptable cost-effective solutions to culturally accelerated eutrophication. Continued surveillance must be incorporated in projects to evaluate actual changes in eutrophy versus predicted changes.

10. To model the microbial effects on eutrophication, a review of existing formulation of microbial activities is required. Changes in bacterial densities in streams have been mathematically described at least since the publication of Public Health Bullentine 143 in July, 1924. The formulations adopted reflected the earlier work of Chick and consisted of composite exponential equations to describe the bacterial die-away.
11. Bacterial growth and accumulation in waste treatment systems have been correlated with substrate energy. The kinetics of this growth has been described by Monod using the enzyme-substrate model of Michaelis. For simplicity linear models are frequently adequate for waste treatment system design because only a small portion of the growth curve is represented.
12. Historically phytoplankton modeling in the engineering literature considered primarily dissolved oxygen relationships. Modeling by limnologists considered nutrient relationships but did not consider symbiotic relationships among bacteria, phytoplankton and zooplankton populations.

13. More recent models evaluate eutrophication by consideration of the bacterial and plankton interaction with organic and inorganic nutrients or other models use statistical relationships among drainage basin use, waste inputs and plankton response.

INTRODUCTION

Pollution and eutrophication originate basically from introduction of organic and inorganic products into the aqueous environment. Man has depended primarily on the abilities of microorganisms to remove these products from the environment through metabolic proclivities inherent only in this biological category. As one advances phylogenetically towards more complex organisms, the inherent ability to react metabolically to a vast array of products is rapidly diminished. The principle of microorganisms in nutrient cycling and biological transformations is presented.

Man, whether he recognizes the fact or not, depends basically on two independent, but not mutually exclusive, processes to cleanse his immediate environment: (1) Oxidation (chemical or photo), and (2) biological processes. The first category with respect to the environment is beyond the immediate control of society. Examples of the second category are apparent in the form of biological waste treatment systems.

Pollution problems have also developed as a result of technology wherein molecules of diverse chemical structures have been synthesized and result in compounds which are refractory to microbial degradation. The refractory nature of many of these chemical structures to biodegradation is well documented in scientific literature. Outstanding examples can be found with detergents, pesticides, and plastics. The inescapable conclusion is that basic ground rules in product development and biodegradability by microorganisms must evolve as direct relationship to molecules entering the environment. A principle of absolute nondegradability for certain structures must be recognized and

established. Historical reference to oxidation ponds, activated sludges, polluted lakes, rivers, and bays attests to the inability of the indigenous microbial flora to effect biodegradation of these compounds. Toxic levels progressively limit the productivity of all higher biological systems creating a polluted aquatic environment.

The terms "eutrophication" and "pollution" need defining since they are often interchangeably and incorrectly used. Pollution from a biological point of view has been defined as an alteration of the environment so as to alter the diversity of aquatic life, eventually destroying the balances in that environment (1). In general, however, pollution means an alteration of the aquatic environment so as to change the beneficial uses. Eutrophication, as originally defined, refers instead to the fertilization of streams (2). Eutrophication is a natural phenomenon which may be aggravated or accelerated by man's activities. Pollution is an unnatural process resulting from the introduction of compounds such as pesticides, heavy metals, mine tailings, and industrial wastes into the aquatic environment. The two terms are not always synonymous.

Excellent bibliographies are available presenting references detailing the effects of nitrogen and phosphorus (3) as well as other nutrients (4) on the aquatic ecosystem. General references detail the effects of excessive fertilization on algae and taxonomic orders of flora and fauna higher than microorganisms (3-6). The specific role of the microorganism in the cycling of elements, sequestering of metals and the effect on solubility of nutrients will be considered here.

PHOSPHORUS

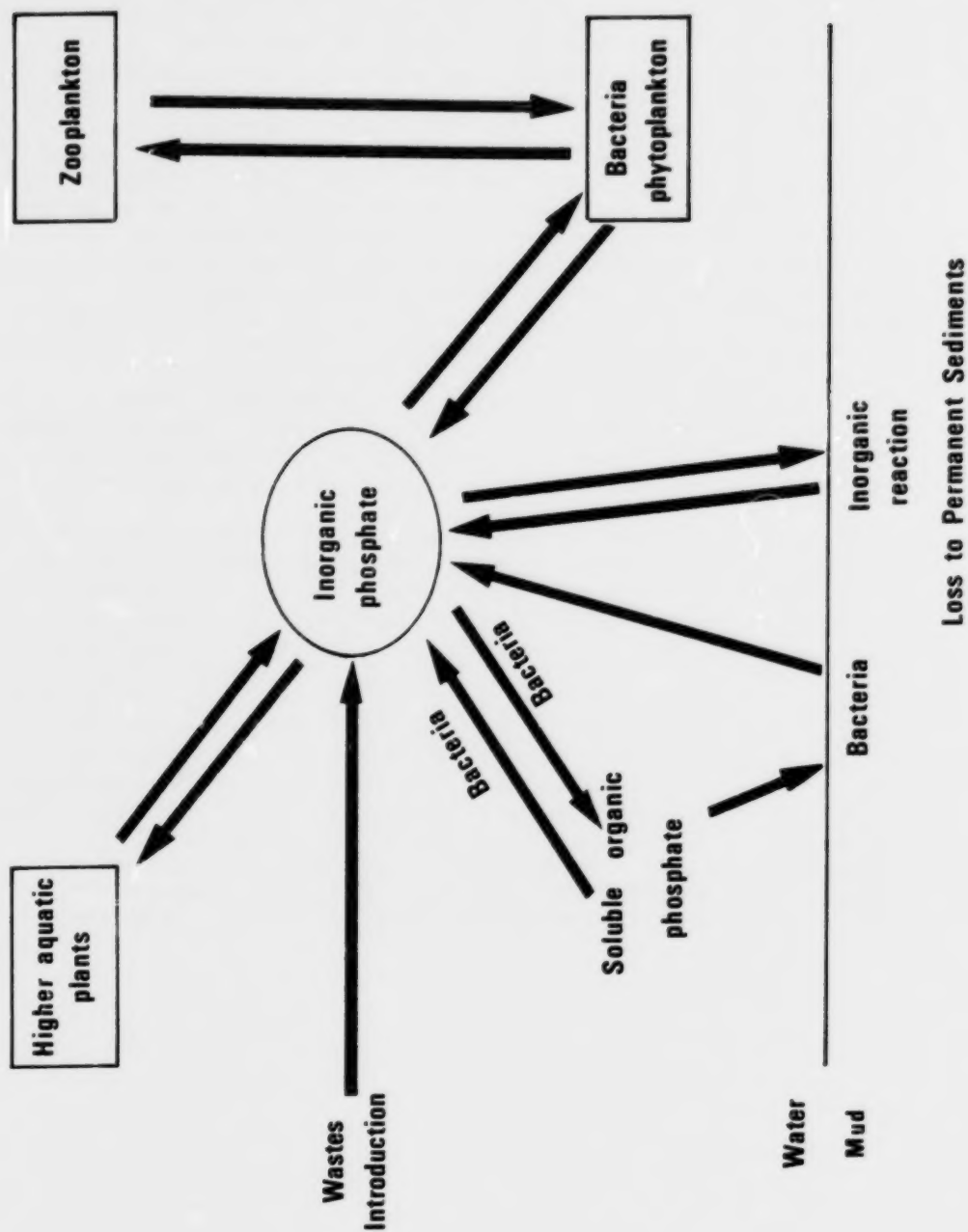
The primary productivity of the aquatic environment may be limited by inorganic nutrients. Phosphorus is one nutrient which can be limiting to the phytoplankton and other organisms. However, most of the phosphorus in the aquatic environment is bound in the sediment as insoluble phosphate salts with availability of the insoluble salts being influenced by both physico-chemical factors (7) and bacterial metabolism (8). The fact that microbial flora influences the availability of phosphate directly through the release of their metabolic end products was recognized by Sackett, et al, in 1908 (9). Soil bacteria utilizing glucose as a carbon source were found to be capable of affecting the solubilization of various insoluble phosphate salts. Later workers demonstrated that the rate of solubilization of phosphate salts was dependent upon the type of carbon and nitrogen sources available to the bacteria (10). The cause-effect relationship between bacterial metabolism and phosphate solubility results from a combination of factors and has been investigated by various workers (9 - 15). Three general processes involved in phosphate solubility are the direct metabolic processes involving enzymes, carbon dioxide production leading to a lowered pH, and organic acid production. A variety of organic acids, which are the end-products of bacterial metabolism, have been shown to increase the solubility of phosphate salts (17,18,19). The mechanism involved may be a chelation like effect.

Another important factor influencing phosphate solubility is the concentration of sulfide. Sperber (20) demonstrated that hydrogen sulfide released soluble phosphate in soils. Hayes and Coffin (21) cite data suggesting that sulfate reduction to sulfide by selected microorganisms could lead to release of

soluble phosphate as a consequence of the conversion of ferric phosphates to the less soluble ferric sulfides. Mackenthun and Ingram (82) also noted that bacteria are capable of utilizing particulate phosphorus, thus making this element available to the rest of the food chains. Biological production of sulfide is limited to two genera, the anaerobic sulfate reducers Desulfovibrio and Desulfotomaculum (29, 30). These organisms also require organic carbon with the release of carbon dioxide and fix elemental nitrogen (31, 32). These findings offer a link between the basic nutrient cycles. Phosphate availability is partially regulated by the same bacteria which affect the sulfur, nitrogen and carbon states in a particular ecological niche. The direct relationship between bacterial metabolic activity and phosphate availability is reinforced by other lines of evidence. For example, changes in dissolved oxygen (DO) have been shown to affect release of inorganic phosphate (PO_4). Anaerobic conditions will cause certain bacteria to release PO_4 , (22) while at high DO concentrations the bacteria concentrate PO_4 in excess of their needs (23).

A cycle is suggested consisting of a algal bloom, subsequent creation of anaerobic conditions, and finally, release of phosphate. Since blooms of algae cause decreased levels of oxygen in water as a result of the decomposition of the biomass, the resulting anaerobic conditions lead to the release of phosphate. As the blooms decline, natural mixing will result in increased DO and the phosphates released from the dead zooplankton and phytoplankton are reabsorbed by the bacteria (22). However, as the nutrients are utilized by the microorganisms, the associated increase in bacterial metabolic activity causes a lower DO and release of phosphates which become available for algal growth resulting in a bloom. This provides an example

Figure 1.
PHOSPHORUS CYCLE



where the eutrophication cycle is intimately associated with and regulated by bacterial metabolism (22,24).

The suggestion that zooplankton are primarily responsible for phosphate regulation in the sea (25) is inconsistent with the data since it has been demonstrated that axenic, or bacteria-free, cultures of zooplankton are unable to concentrate phosphate or to use organic phosphate (26, 33, 34). Further, the zooplankton were found to supply only one-third to one-tenth of the phosphate necessary for phytoplankton (27). Thus the bacterial systems appear to be a major agent in the regeneration and solubilization of phosphate in aquatic environments. Conclusive demonstration of direct biological exchange of phosphate is difficult, as most of the activity occurs below or at the sediment surface. However, it has been shown that the bacteria are at least partially responsible for the exchange of phosphates, both organic and inorganic in the water (28). Nevertheless, as seen in Fig. 1, a generalized scheme for phosphate cycling in an aquatic environment has been suggested.

Phosphate turnover or regeneration in aquatic systems at the sediment-water interface is greatly influenced by bacterial metabolic activity. The bacteria are capable of both sequestering phosphate in excess of their needs, and at the proper stimulus, release the phosphate (8). Aquatic bacterial populations can degrade organic phosphates to inorganic phosphates and influence phosphate solubility as a consequence of their metabolic end-products. As Hayes and Phillips (8) have shown, addition of antibiotics to the aquatic system will greatly reduce phosphate availability. The data all point to the need for a more complete understanding of the microbial process as related to phosphate regeneration and the influence other nutrients exert on phosphate availability. However,

the actual extent of these reactions and the effects of various pollutants in the form of pesticides, heavy metals, industrial acid and alkali wastes on the bacterial flora as related to the solubility of phosphate is unknown.

SULFUR

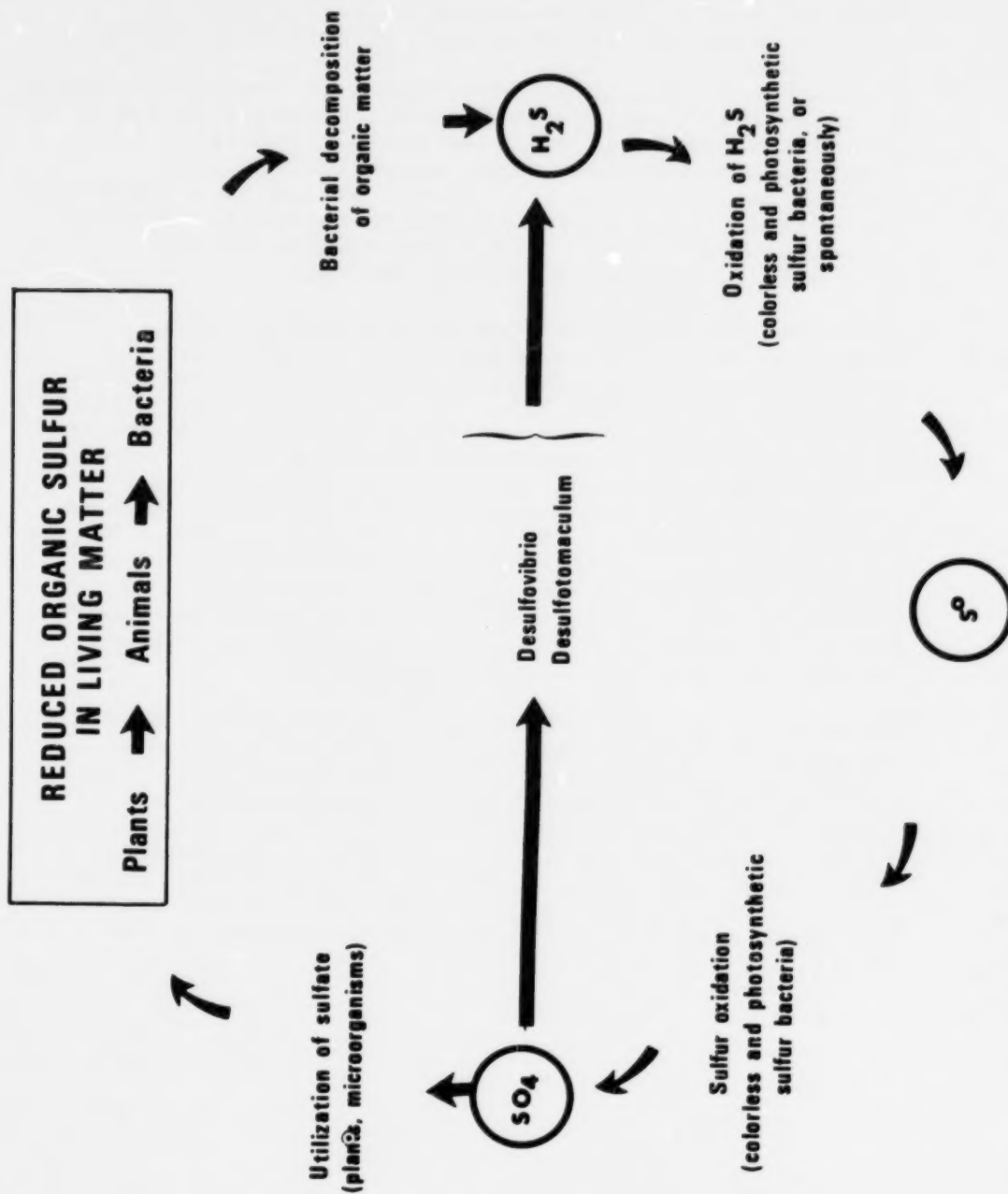
Biological cycling of carbon, nitrogen and phosphorus and the importance of these elements to the fertility of soil and water is widely recognized. Nevertheless, other elemental nutrients undergo similar cyclic processes and are of equal importance to the fertility of the ecosystem. One of the most easily definable cycles in nature is the sulfur cycle. Although not widely recognized, the availability of sulfur can limit productivity of the aquatic environment and has been linked to decreased productivity of fish (67).

The biochemical and physiological events resulting in the transition of sulfur through the various valence states have been the subject of many reviews (35-38) and will not be considered here. Rather, the availability of sulfur in the aquatic environment as regulated by microorganisms will be considered. The dynamics of sulfur cycling through the gaseous, liquid, solid, organic and inorganic compounds, oxidized or reduced states, can be represented by a cycle as shown in fig. 2(39-43).

There are a variety of reactions involved in the sulfur cycle and these can be divided into two principal pathways: 1) the reduction of sulfate to sulfide and 2) the oxidation of the reduced compounds to sulfate (Fig. 2). Biological oxidation and reduction of inorganic sulfur compounds in nature is the result of a relatively select group of ubiquitous microorganisms (44). Chemical reactions may also occur because of the

Figure 2.

THE SULFUR CYCLE



relative instability of certain sulfur compounds (45), however, these chemical reactions occur exclusively with the highly reduced states of sulfur such as sulfide resulting in deposition of elemental sulfur.

The extent of microbial involvement in the sulfur cycle is evident to even the most casual observer by the wide spread production of hydrogen sulfide resulting in black muds and soils. Also, the extremely acid conditions found near acid mine drainage is a result of the metabolism of sulfur oxidizing bacteria. Both of these phenomena are a direct consequence of the metabolic processes of sulfur bacteria.

Microbial oxidation of reduced inorganic sulfur compounds in nature is mediated by one of three different ways: 1) oxidation by "colorless sulfur bacteria" i.e. Thiobacilli 2) by the photosynthetic sulfur bacteria, and 3) incidental oxidation by various heterotrophic or autotrophic microorganisms. The relative importance of the chemical oxidation and of the three microbial processes is discussed by various workers (41,46,47). There is presently no means for in situ measurement of the actual contribution of each process in sulfur oxidation (48). However, it is generally agreed that the "colorless sulfur bacteria" are of greatest importance for oxidation of reduced sulfur compounds in nature (49,50,51).

Examples of the colorless sulfur bacteria are the Beeggiatoa and Thiothrix which are filamentous and associated with polluted waters and found in polluted waters. Both of these organisms can be found easily in ponds which have become polluted by either sewage, fallen leaves or other organic wastes. Presence of these organisms is evidenced by the white scum which often is sulfur which forms on the top of the water. Specialized sulfur oxidizers are also found in sea

waters and are grouped into the genera Thiovulum and Thioploca (68). These specialized aerobic sulfur oxidizers are responsible for the biological oxidation of sulfide to sulfur.

The anaerobic oxidation of hydrogen sulfide to sulfur is also mediated by certain photosynthetic bacteria which can be divided into the red Chromatium and Thioploca and green or Chlorobium and Chloropseudomonas. The red and green sulfur bacteria are capable of an anaerobic photosynthesis coupled to the oxidation of sulfide to sulfur. The red sulfur bacteria may form large zooglycal masses. Carbon dioxide and nitrogen are fixed by various members of these two groups (68). Other sulfur oxidizing bacteria are capable of oxidizing elemental sulfur to sulfate. The colorless sulfur oxidizers, the Thiobacilli, are able to oxidize a variety of reduced sulfur compounds both in aerobic and anaerobic conditions, and to use either organic carbon (heterotrophic growth) or carbon dioxide (autotrophic growth) (49,52). Representative species of Thiobacilli have been isolated from a variety of aquatic environments such as sea water (53), fresh water (54), marine sediments (55), and soils (56). Thiobacilli are especially abundant in estuarine waters and sediments (57,58) and in volcanic sulfur springs (59,60,61).

Biological reduction of sulfate can proceed by one of two separate pathways, assimilatory and dissimilatory (35). The assimilatory pathway involves the incorporation of sulfate into organic compounds and is carried out by the majority of bacteria, fungi, yeast, algae and plants (see 44). The dissimilatory pathway involves the direct reduction of sulfate via sulfite to sulfide. In this pathway, the function of sulfate is analogous to that of oxygen in aerobic systems in that sulfate is the terminal electron acceptor.

Dissimilatory sulfate reduction is a property exclusive of two genera of ubiquitous microorganisms the Desulfovibrio (30) and Desulfotomaculum (29). The dissimilatory sulfate reducers have been found in marine environments (69,70), Antarctic waters (71,72), fresh water (73), inland lakes (74), and soils (75,76).

In a balanced ecosystem, the relationship between the sulfur oxidizers and sulfate reducers is an intimate one. The sulfur oxidizers yield sulfate and are capable of autotrophic growth fixing carbon dioxide to yield organic carbon. The autotrophic sulfur oxidizer Thiobacillus denitrificans also reduces nitrate to nitrogen (77). The sulfate reducers have been found to fix elemental nitrogen (78,79,80,81), reduce sulfate to sulfide and require organic carbon for growth (35). Hence, the microbial activity which is associated with the sulfur cycle influences the cycling of other essential nutrients.

Although animal systems can oxidize reduced sulfur compounds such as sulfide (62), thiosulfate (63), and sulfite (64), and can incorporate sulfate into various organic molecules (65), they are unable to reduce sulfate to sulfide (66). To accomplish this they must depend upon plants and bacteria to provide them with reduced sulfur compounds (33, 35). Thus it becomes apparent that the sulfur cycle, as regulated by microbial life, influences aquatic life not only by making sulfur available in various oxidation states, but also affects nitrogen, carbon, and phosphorous concentrations.

NITROGEN

Biological nitrogen cycling involves, as does cycling of sulfur and phosphorus, the transition of an

elemental nutrient through various chemical states. It is convenient to initiate consideration of the nitrogen cycle at the point where the fixation of gaseous nitrogen occurs, i.e., where atmospheric nitrogen is converted to ammonia. Relatively few species of microorganisms populating the earth are capable of metabolizing nitrogen from the air. Among the first investigators contributing to an understanding of the role of symbiotic microorganism in leguminous plants was Beijerinck (87). His work has been continued and expanded by many, and the history of our understanding of the biology of bacterial nitrogen fixation in leguminous plants has been thoroughly reviewed (89). We now know that the only organisms capable of nitrogen fixation are certain free-living and symbiotic bacteria and blue-green algae (85,86,88,90). The biological transformation of molecular nitrogen through its inorganic chemical states by this select group of microbes makes nitrogen available to all the world's flora and fauna which are dependent, either directly or indirectly, upon these microorganisms.

The fact that nitrogen fixation is unique to a few species of bacteria and blue-green algae, gives rise to important ecological questions. Among the most challenging are those relating to discovery and elucidation of the mechanisms of biologic nitrogen fixation. It was not until 1960 that cell-free fixation was demonstrated using extracts prepared from the anaerobic bacterium Clostridium pasteurianum (91, 92). This fundamental break through has permitted a rapid expansion of our knowledge concerning the biochemical mechanisms involved in nitrogen fixation, and many reviews are available detailing the physiological processes carried out (85, 86, 88, 93, 94). In several respects, it is now clear how nitrogen gas can be utilized as a source of nitrogen for the synthesis of amino acids, nucleic acids and other

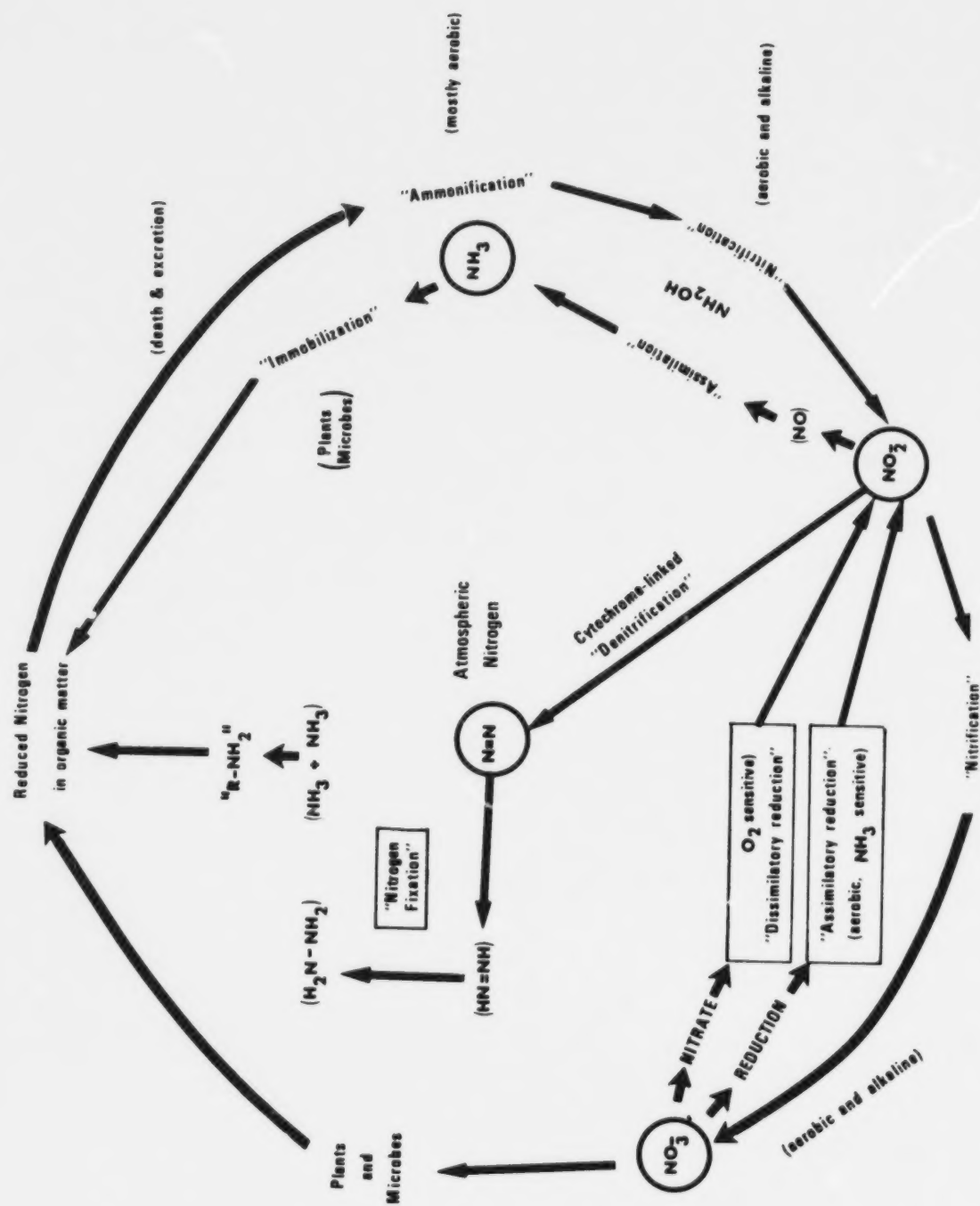
nitrogen-containing cellular components by these microorganisms, and how the nitrogenous compounds are transferred to higher plants and animals along the food webs in nature and in agriculture.

Another striking aspect of nitrogen cycling is the variety of the effects that alterations in the quality and quantity of organic carbon and concentrations of phosphorus and sulfate have on the activities of the microbes involved in nitrogen transformations.

It has been demonstrated that increased availability of an easily utilized carbon source like glucose will generally depress the amount of atmospheric nitrogen fixed in the aquatic environment. In contrast, increasing availability of soluble phosphorus will tend to increase nitrogen fixation (95). The biochemical bases for these phenomena apparently are found in the complex nutritional relationship of the various heterotrophic organisms responsible for nitrogen fixation and their requirement for various carbon substrates, and the interactions among the enzymatic processes involved.

The activity of the organisms responsible for sulfate reduction and sulfide oxidation will affect both nitrogen fixation and denitrification (32, 75, 77, 80, 81). Increased sulfate concentrations in an ecosystem will enrich the capacity for outgrowth of sulfate reducing bacteria which fix nitrogen. Furthermore, as a consequence of the hydrogen sulfide evolved by these bacteria, a drop in eH will occur. The lower eH favors the development of other anaerobes capable of nitrogen fixation and in the denitrification reactions as well. Neither phenomenon can be viewed independently of the other.

Figure 3.
REVIEW OF THE NITROGEN CYCLE



The availability of oxygen in aquatic systems affects the operation of the processes of denitrification, nitrification. These reactions are illustrated in Fig. 3 and are the major components of inorganic nitrogen metabolism. Depending upon the various chemical and physical parameters impinging on a system, there may be increased or decreased quantities of nitrate, ammonia and in rare instances, nitrite in the aquatic environment.

It has been suggested that the overall contribution of fixed nitrogen by bacterial and blue-green algae in certain fresh water lakes amounts to 15 mg per square meter in a day (95). While this appears to be inconsequential when compared to the contribution that is due to agricultural runoff, the total in situ microbial fixation of nitrogen in a Wisconsin lake has conservatively been estimated to be in excess of 80,000 lbs per year (178). This amount of fixed nitrogen is thought to be introduced into the aquatic environment during certain seasons and is exclusive of any excess which may be introduced due to agricultural or sewage runoff.

Subsequent to fixation the relative concentrations of the inorganic nitrogen compounds in water, i.e., nitrate, nitrite, and ammonia, depend, in part, on the amount of oxygen available and the oxygen concentrations are dependent upon the organic carbon load and seasonal variations in solubility of oxygen in water. Attempts to develop a nitrogen balance in lakes and other aquatic environments are hampered by the fact that there are several possible sources for loss of nitrogen. For example, fixed nitrogen can be lost via: (1) lake effluents; (2) loss of volatile nitrogen such as ammonia and nitrogen gas (3); denitrification by certain microbes; (4) precipitation of nitrogenous compounds into either permanent or

semipermanent sediments; and (5) removal of organisms by fishing, weed harvesting or other methods of flora or fauna depletion (95).

In general, well oxygenated waters will contain higher concentrations of nitrate than ammonia and very little, if any, nitrite. One possible reason for this is the action of the nitrifying bacteria which will aerobically convert ammonia to nitrate autotrophically. However, in waters containing lower oxygen concentrations there will be higher concentrations of nitrite and ammonia (96, 97, 98). During summer months, inorganic nitrogenous compounds may completely disappear from the epilimnion in some lakes even though abundant in spring (98).

The biochemical mechanisms involved in denitrification have only recently been elucidated in significant detail (99-102). Those reactions that result in the conversion of nitrate to nitrogen gas are unique to a limited group of microorganisms found in the family Pseudomonadales.

A generalized interrelationship between the concentrations of ammonia, nitrate, nitrite, and oxygen in various systems can be developed based on the data from both laboratory and field studies. It has been shown that oxygen will inhibit denitrification (nitrate to nitrogen) in Pseudomonas perfectomarium, an aerobic marine bacterium. However, when the oxygen level becomes limiting for aerobic growth these aerobic organisms can use either nitrate or nitrite as an electron acceptor, that is, as a substitute for oxygen (99-102), in their respiration. It has been found that in lakes and in the ocean, nitrate concentrations follow temperature or seasonal variations and vary with depth (95, 106-108). The oxygen levels also parallel

these variations so that as the oxygen concentration falls, the organisms begin to utilize nitrate as a terminal electron acceptor. The result is nitrate concentration reduction or depletion. The field results are in agreement with laboratory studies of Payne, et al, (99-102). By the process of nitrification (ammonia to nitrate), respirable oxygen in the form of nitrates becomes available as a stored oxygen resource to be liberated by the denitrification process (109).

Although findings from both laboratory and field studies are consistent and tend to support the above conclusions (95, 99-109), it must be noted that there have been no definitive studies which actually link all of the above detailed conditions at the same location and time. Nevertheless, the above relationships serve further to emphasize that all the nutrients are co-equal in importance and any alteration in components of one cycle can affect other nutrient availability. For natural populations in aquatic systems, the theory of nutritional control by a "master nutrient" is not consistent with data obtained from laboratory experiments and from field observations.

CARBON

The carbon cycle is composed of an integrated network of physical and biologically mediated pathways encompassing the synthesis, degradation, and transformation of innumerable simple and complex organic molecules. Superimposed on the carbon cycle are the controls exerted by nutrient availability, and the fixation and evolution of carbon dioxide. Various aspects of organic carbon cycling in the aquatic environment have been examined (103, 104) with the emergent principle that an overall balance between production or synthesis and decomposition of naturally

occurring substances exists in nature. Photosynthetic carbon dioxide fixation by green plants is the major route by which carbon enters the organic carbon cycle. However, fixation by autotrophic bacteria add to the total carbon budget in the ecosystem (120,121). Once organic material has been introduced into the aquatic environment the endogenous flora and fauna can either utilize or contribute to, depending upon conditions, an existing reservoir of organic material(105). Some ecological questions relating to carbon arise when considering the microbes direct relationship to carbon cycling. For example, what affect does microbial synthesis of complex molecules such as vitamins, amino acids, carbohydrates and lipids have on the aquatic biota; what is the contribution of the bacterial biomass as a food source for zooplankton; and, what is the significance of microbial degradation of suspended, soluble and sedimented organic compounds? The health related aspects of bacterial growth are of importance. Growth of known pathogenic microorganisms can result from the introduction of raw sewage into water. Further, recent studies demonstrated that microorganisms normally considered of no medical significance are capable of causing human illness (122). The introduction of wastes from citrus processing plants and waste paper pulp mills into rivers and lakes has been demonstrated as providing an environment suitable for the growth of bacteria capable of causing human disease. These complex interrelationships of bacteria to the aquatic habitat can affect water quality from a public health point.

Another dimension is added with respect to carbon cycling by the addition of synthetic organic compounds. Examples of such synthetic compounds are certain pesticides, detergents, and various other industrial materials. The majority of the biodegradation process

involving these compounds are achieved exclusively by the bacterial population.

Direct and complex relationships between diverse organisms have evolved based on the needs for various growth factors. Examples of these relationships are seen in the association of various algae and bacteria in the marine and fresh water environment (110-112, 119). Apparently, the microbial flora is one of the major producers of surplus vitamins which stimulate the growth of other algae (113-118). In practical terms, the reason that various eutrophic agents, such as excess phosphates, sulfate, nitrate and certain carbon compounds, cause characteristic nuisance blooms can be traced to the abundant growth of certain bacteria which produce the necessary growth factors for algae (110).

In addition to providing essential growth factors, the bacteria further serve as a source of food for higher aquatic organisms. Specific marine invertebrates were shown to consume bacteria, as well as require a select microbial flora to aid in their digestion (123). Bacteria and protozoa adsorbed onto detritus also provide nutrition to certain fish (124). Marine benthic organisms such as bivalves and bottom feeding fish have been shown to consume bacterial cells as a food material (125). A generalized scheme becomes apparent. Bacteria and fungi initiate the primary stages of oxidation, hydrolysis and assimilation of the carbon structure of plant debris. The bacteria are consumed by protozoa creating a bacteria-protozoa-detritus system which serves the role of a food source for a larger organisms such as snails, bivalves and fish. Such cycles have been suggested as providing food sources under controlled conditions (124).

Credence is given to the above suggested cycle since the bacteria are responsible for the

decomposition of nutrient organic matter and the bacterial biomass in certain marine environments is equivalent to that of the benthic organisms (126). Bacteria have also been shown to act as concentrators of nutrients in dilute solution. Protozoa, for example, grow poorly in a dilute nutrient medium but flourish in the presence of bacteria (127). Brine shrimp have been shown to grow only over sediments which support a bacterial-protozan population and not over a particulate sediment (128, 129), and are apparently dependent upon the bacterial populations.

An interesting relationship between nutrient availability and bacterial growth has been observed in the presence of excess phosphate ions. Bacteria are incapable of utilizing organic carbon material adsorbed onto clay particles. However, once the clay was exposed to phosphate ions (0.3M concentrations) the organic material became desorbed and the bacteria were able to multiply (130). This fact suggests a second role of phosphate in water in addition to constituting an essential mineral element for growth. Physical factors involving phosphate ions and the solubility of organic compounds are of importance in determining the primary and secondary role of phosphate as a nutrient.

Soluble organic material in waters is composed of free sugars, (see 131, 135), and amino acids (132-135) where a relationship appears to exist between these organic nutrients and heterotrophic bacteria (135-137). Both bacteria (139) and fungi (140) are responsible for the decomposition of the soluble or suspended organic material and the higher aquatic plants and benthic organisms are responsible for the primary production (141,142). Of all the soluble or suspended organic material in the water column, it has been suggested that only 10-20 percent reaches the bottom (145) with 80 to 90% being consumed by bacteria in the upper

layers. Physical factors, including the height of the water column and turbulence, will affect the sedimentation rates making it impossible to extrapolate results between bodies of water due to variations in the physical environment (144).

Soluble organic material does adsorb onto clay particles at the sediments by mud water interface (130). However, the adsorption may not be permanent and an equilibrium is reached between the adsorbed and soluble material (145). Thus, the clay acts as a potential nutrient sink and the associated bacterial activity creates an appreciable oxygen demand (144). Consumption of oxygen at the bottom creates an anaerobic benthic environment which favors the growth of the methane producing and other anaerobic bacteria. A vigorous anaerobic bacterial activity is evidenced by the fact that methane hydrogen sulfide, and carbon dioxide has been found coming up from lake bottoms (146,147). The bacteria, fungi, and heterotrophic fauna in the sediments utilize the biological debris and act as major recyclers of dead fauna and flora.

The importance of microbial activity in decomposition of biological debris is best exemplified in certain bogs. When the water quality is such that pH and dissolved oxygen become limiting factors to bacterial growth resulting in decreased biological activity, a corresponding accumulation of peat occurs (plant and animal debris). However, this build up can be reversed if the physical conditions are controlled allowing a resumption of bacterial metabolic activity (148-150). Thus the role of the microbes (bacteria and fungi) is concerned directly with the breakdown and cycling of the relatively resistant skeletal material of plants and animals. It has been suggested that bacteria maintain the organic material at low concentrations

TABLE 1

Biodegradable Compounds*

ORGANIC COMPOUND	DEGRADING MICROBIAL ORGANISMS	REFERENCE
1. Phenol	<u>Bacillus</u> , <u>Micrococcus</u> <u>Pseudomonas</u> species	152
2. Chlorophenoxyethyl sulfate	<u>Bacillus cereus</u>	156
3. Long-chained alcohols C12-C20	<u>Pseudomonas</u> C12B	157
4. Phenoxyalkyl carboxylates	<u>Flavobacterium</u> species	158
5. Primary alcohol sulfates	<u>Pseudomonas</u> C12B <u>Aerobacter cloacae</u>	159,160
6. Secondary alcohol sulfates	<u>Aerobacter cloacae</u> , <u>Pseudomonas</u> C12B	161
7. Ether glycols	Bacterium TEG-5	153,162
8. Chloroacetate; dichloroacetate	<u>Pseudomonas</u> species	163
9. 4-Chloro-2-methyl phenoxyacetate	<u>Flavobacterium peregrinum</u> , <u>Arthrobacter</u> species	164
10. Straight-chained isomers of alkyl benzene sulfonate	<u>Pseudomonas</u> C12B	154,155
11. Polyvinyl alcohol	<u>Pseudomonas</u> species <u>Bacillus mesentericus</u>	165
12. Trifluralin	<u>Pseudomonas</u> species	166
13. Aresin	<u>Bacillus</u> , <u>Pseudomonas</u> species	167
14. Phenyl mercuric acetate; ethyl mercuric phosphate; methyl mercuric chloride	<u>Pseudomonas</u> K62	168
15. Prometryne	<u>Aspergillus</u> species	169
16. Acetonitrile	activated sludge	170
17. Nitrobenzoates	activated sludge	171
18. Chlorinated alkyl and aryl compounds	activated sludge	172

*Adopted from Payne, et al. (151).

(138) and are exclusively responsible for the uptake of nutrients even in high concentrations (136).

Assimilation and biodegradation of naturally occurring organic compounds as a dynamic biological continuum of activity is tacitly assumed and accepted. When natural bacterial processes become inhibited, a build-up of organic debris leading to the creation of bogs. As man has progressed culturally and technologically, new stresses have been placed on the environment in the form of man-made, synthetic organic material which may or may not be biodegradable. Build-up of the non-degradable agents, such as certain non-linear alkyl sulfonates (151) and certain pesticides, are of general concern since they accumulate in the environment.

Fortunately, most synthetic compounds are subject to bacterial degradative processes (see table 1). Microbes, however, are not infallible and are incapable of degrading certain halogenated organic molecules. Examples of persistent compounds are the pesticides aldrin, dieldrin, endrin, toxophene, DET, benzene hexachloride, chloradene, and others (173). These compounds are not a part of the nutrient cycle since they do not provide an energy source. However, molecules can be concentrated by microbes (174, 175, 176) providing one entry point into the food cycle for further degradation or deposition in sediments. Bacteria, as noted above, do provide a food base for many benthic animals, and as has been suggested are a major source of food for aquatic fauna (177). of naturally occurring substances exists in nature.

ENGINEERING ASPECTS

The engineering phase of any study, stated simply, is the evaluation of all available scientific information and subsequent determination of the optimum control

plan within political, economical and scientific constraints to obtain an objective. By this definition most papers discussing the control of eutrophication by limiting some essential nutrient are engineering oriented.

The basic steps in controlling the eutrophication of a lake for example are:

1. Determination of the source of nutrients and the biological response of the system to those nutrients by means of field surveys and laboratory studies.
2. Analysis of the information by mathematical models or other methods which include consideration of alternative control schemes and which incorporate economic factors in addition to scientific factors.
3. Implementation of the control scheme by facility construction and/or system modification and operation.
4. Evaluation of the effectiveness of the solution by surveillance of water quality changes.

Items 1 and 2 in the above listing actually are interrelated and occur simultaneously in well conceived projects. Mathematical models must be adapted to or developed for a particular waterway to take into account geomorphological characteristics. Data collection likewise must be tailored to supply proper input to such models.

The implementation phase is the classic engineering design and construction of structures such as sewage

treatment facilities with nutrient removal or the installation of sewers to collect direct discharges for treatment or diversion out of a drainage basin. Operation of these facilities to maximize nutrient removals is becoming more important.

Surveillance is an often overlooked phase but which is extremely important, especially currently when large sums of money are to be spent for nutrient removal. It is important to study and compare actual lake water quality changes versus those predicted and to establish true cost per improvement amount.

Engineering Analysis

The primary interest of this report is the microbial cycling which has been described for the various inorganic nutrient cycles. To tie these cycles together and predict biological production at various levels in the ecological system is one approach to determining the critical nutrient for a given system. Another approach is statistical and consists of correlating land use patterns and nutrient runoff loads versus various measures of eutrophy. By calculating various reductions in loading corresponding reduced levels in the eutrophy indicators occur.

Modeling Bacterial Systems

The mathematical modeling of bacterial systems described in the sanitary engineering literature generally concern either descriptions of bacterial densities downstream from waste discharges or the increases in the mass of organisms in treatment systems.

Public Health Bulletin No. 143 (179) which reports on an extensive water quality study of the Ohio River

contains one of the first attempts of quantitatively describing bacterial behavior downstream from waste inputs in a natural system. Two types of formulations were fitted to the data collected from the Ohio River. The first curve was the autocatalytic or logistic function:

$$y = \frac{b}{1 + (ct + d) 10^{at}} \quad (1)$$

where y = bacteria as a percentage of the maximum density

t = time of flow from sewer outfalls

a, b, c, d = constants required to fit data points
The second formulation was a composite exponential type formulation:

$$y = a(10^{-bt}) + c(10^{-dt}) \quad (2)$$

where the symbols are the same as above. Equation (1) was fitted to crested shape curves which were indicative of a growth phase before a die-way phase. Equation (2) was simpler to evaluate and was used to fit only the declining portion of the curve.

Equation (2) is actually an extension of results attributed by Streeter (180) to Chick for describing bacterial mortality caused by disinfectants. The equation attributed to her work was:

$$\frac{1}{t_2 - t_1} \log y_1/y_2 = K \text{ or } y_1 = y_2 \exp(-K(t_2 - t_1))$$

y₁, y₂ = numbers of live bacteria

t₁, t₂ = time

K = proportionality constant

However, this simple formulation was inadequate to explain the Ohio River die-away curves and led to the formulation of the composite exponential mathematical form to explain the die-away portion of the curve.

Subsequently, Streeter (180) developed a composite exponential to explain the crested shaped curve as well as the die-away portion during the Ohio River Studies.

The equation developed was of the form:

$$y = (y_a - b_a) 10^{-k+n} + [(b_a - C_a) 10^{-k_1 t} + C_a 10^{-k_2 t}] \quad (4)$$

where: y =denotes numbers of bacteria

t =time

y =initial bacterial numbers

a, b, c =constants

n =constant which determines the sharpness of the crest

k =reaction constant

The values of all constants were determined graphically.

Hoskins (181) analyzed bacterial data collected from the Ohio River and the Illinois River. For these data, two sets of die-away curves were developed to distinguish between summer (T 15 C) and winter (T 15 C) conditions. Each set contained curves which were proportional to the initial bacterial density.

It is interesting to note that these early workers expended as much effort describing the bacterial densities associated with pollution as with the oxygen sag formulations. However, only the latter formulations came into general use by sanitary engineers.

Fair and Geyer (182) suggested another formulation which is of Chick's law and includes a non-uniformity coefficient. Their equation is in the form of a retardant and is written:

$$N/N_0 = (1+nkt)^{-1/n} \quad (5)$$

where: N = the modal number of bacteria in a stream at time zero

N = the modal number of bacteria at subsequent times downstream

K = is the initial die-away rate constant

n = coefficient of non-uniformity

It can be shown that equation (5) becomes equation (3) when $n=0$ by evaluating the indeterminate form that results using the mean value theorems of the calculus (183).

Much of the early work concerned bacterial groups other than the coliform bacteria. Streeter's work considered additionally the enumeration of standard plate counts on nutrient agar and on gelatin (179). The agar counts were incubated at 37°C and were considered indicative of heterotrophic bacteria acclimated to the bodies of warm blooded animals. Gelatin plates were incubated at 20°C and were considered indicative of heterotrophic bacteria favoring locations in nature outside of warm blooded animals such as soils. Overlap between these two groups were recognized but the extent was undetermined (179).

None of these formulations considered bacterial numbers as part of the overall food chain. Streeter (180) presented simplified calculations which alluded to the relationship between a given number of bacteria and the

BOD observed. His analysis was limited to the heterotrophic oxidative assimilation of organics. Although Streeter (184) recognized the phenomena of nitrification, he did not include the effect in the oxygen sag formulation because of the belief that it occurred after the critical point (maximum dissolved oxygen deficit) of the sag curve was reached.

Others in the U.S. Public Health Service Cincinnati group (185, 186, 187) in addition to Streeter recognized and described the bacterial-zooplankton relationships in natural systems. Butterfield, et al. (186) presented both bacterial density and BOD data as a function of the presence or absence of zooplankton. In fact, bacterial regrowth in streams was postulated as resulting from an interruption of the bacteria-predator relationship (187). This phenomenon was not explicitly formulated in any of the descriptive equations for bacterial survival presented although the effect was implicitly included in the various die-away coefficients. The increase in biological mass (sludge) for treatment systems of the activated sludge type is a significant design parameter. To accomplish prescribed treatment in terms of organic constituent removal, treatment systems must provide a suitable environment in terms of waste-sludge contact time, sedimentation time and sludge wasting facilities. Pilot plant empirical determinations of these parameters are as old as the activated sludge process itself. An excellent literature review of the systematic study of sludge production has been prepared by Servizi and Bogan (188).

Servizi and Bogan (188) suggest that the amount of synthesis of activated sludge is proportional to the free energy of the substrate. Laboratory evaluations of the proportionality constants yielded the following equations:

carbohydrates $y = 0.38 \text{ COD}$ (6)

aromatics & aliphatic acids: $y = 0.34 \text{ COD}$ (7)

where: y = gross synthetic yield in grams of
cells per mole of substrate

COD = chemical oxygen demand by dichromate
oxidation in grams per mole of substrate

The concept of bacterial growth yields as a function of substrate energy was further validated by the work of Mayberry, et al (189). They calculated yields by four different methods: grams of cells per mole of substrate, gram atom of carbon used, mole of oxygen consumed, and equivalent of "available electrons" in the substrates. The "available electrons" calculation or energy available system proved the most consistent.

Although the amount of sludge produced could be determined, the rate of production still needed quantification. For simple pure culture systems using a single substrate, Monod (190) used the enzymatic kinetics of Michaelis as a model to predict rates of growth. Such kinetics are the generally accepted model today for purposes of modeling biological systems. For mixed cultures such as activated sludge which oxidizes mixed substrates of varying complexity, simpler kinetic models have been adopted.

For example, Eckenfelder and O'Connor (191) adopted a linear model:

$$\Delta s = a \cdot r - b S_0 \quad (8)$$

Where: Δs = increase in sludge concentration
 r = the BOD (or COD) removed per day
 S_0 = the initial sludge mass per unit volume
 a = fraction of BOD removed synthesized to new sludge per day (see constants on equations (6) & (7))
 b = rate of endogenous respiration expressed as a fraction per day

This model proved adequate for design when the system operated over only a small portion of the growth curve (e.g. declining growth - endogenous respiration).

All of the foregoing bacterial models assumed that sufficient growth factors and trace elements are present. Very little work has been done to determine what critical levels of trace elements are required. Eckenfelder and O'Connor (191) reported that other than nitrogen and phosphorus, trace elements are generally available in sufficient quantities in natural waters. Helmers, et al. showed that for optimum activated sludge process efficiency a minimum nitrogen and phosphorus content of the sludge expressed as a percent of the total volatile solids is 7 percent and 1.2 percent respectively. This same study indicated that the critical amounts of nitrogen and phosphorus per 100 lbs. of BOD removed were 3-4 lb and 0.6 lb. respectively (cited in 191).

Algal Modeling

Mathematical modeling of algal systems in the engineering literature has been concerned predominantly with net oxygen production. Oxygen sag formulations of the Streeter-Phelps type frequently include the terms photosynthetic oxygen production (P) and algal oxygen respiration (R). The difference between production and respiration is the net oxygen production which may be positive or negative. Several authors (192, 193, 194, 195) have presented models which include such phenomena. Such models are solved for steady state conditions after the algal mass has developed. No consideration was made of the growth phase or the die-away phase of these algae. Inorganic nutrients were therefore not incorporated into the formulations. Additionally, such formulations describe the effects caused by the algae rather than the kinetics of the basic algal processes involved.

Limnologists have produced sophisticated mathematical productivity models of two basic types. The first type considers the dynamics of growth in relation to growth factors. The second type considers productivity in relation to depth of the water column. These distinctions and descriptions of the various developments are presented in an excellent review of the subject prepared by Patten (196). Patten presented a qualitative model which has terms considering the major factors affecting plankton populations:

$$\frac{\partial B}{\partial t} = \text{income} - \text{loss}$$

$$= [f_1 (\text{influx of producer biomass}) + f_2 (\text{photosynthesis}) + f_3 (\text{influx of higher trophic level biomass}) + f_4 (\text{auxo-trophic and heterotrophic growth})] - [f_5 (\text{respiration}) + f_6 (\text{grazing}) + f_7 (\text{efflux biomass from all trophic levels})] \quad (9)$$

where B = plankton biomass in a unit volume of water
 at some depth Z
 t = time
 f(i), i=1, 2...7 are processes expressing

Each of the subsequent models presented by Patten considered various combinations of terms of equation 9, as well as different functional relationships for these terms. Many of these relationships can be used in the development of control strategies. These models, however, do not consider symbiotic relationships between bacteria, phytoplankton and zooplankton populations.

Eutrophication Control

Recently, several researchers have developed mathematical models which attempt to describe phytoplankton population dynamics by consideration of nutrient inputs and bacterial metabolism specifically for the purpose of providing a basis for developing eutrophication control programs through nutrient input limitations.

Two types of models are available. The first type describes algal dynamics using kinetics such as those described by Patten (196) in his review. The second type uses statistical procedures to analyze nutrient inputs and lake responses from many lakes by applying multiple regression statistical techniques. The resulting equations are used to predict eutrophic conditions in homologous lakes by incorporating the input of the requisite nutrient data with other physical and chemical information.

Examples of the first type mathematical model have been developed by Echelberger, et al. (197), Di Toro, et al. (198), and Water Resource Engineers, Inc. (199). Examples of the second type include the work of Vollenweider (200) and that of Brezonik and Shannon (201).

Table 2

First Principal Components (y_{CO} and y_{Cl})
and Trophic State Indices (TSI_{CO} and TSI_{Cl})

(a) Colored Lakes:

$$y_{CO} = .848(1/SD) + .809(COND) + .887(TON) + .768(TP) \\ + .930(PP) + .780(CHA) + .893(1/CR)$$

Cumulative Percent of Total Variance Explained by y = 72%

$$TSI_{CO} = y_{CO} + 9.33$$

(b) Clear Lakes:

$$y_{Cl} = .936(1/SD) + .827(COND) + .907(TON) + .748(TP) \\ + .938(PP) + .892(CHA) + .579(1/CR)$$

Cumulative Percent of Total Variance Explained by y_{Cl} = 71%

$$TSI_{Cl} = y_{Cl} + 4.76$$

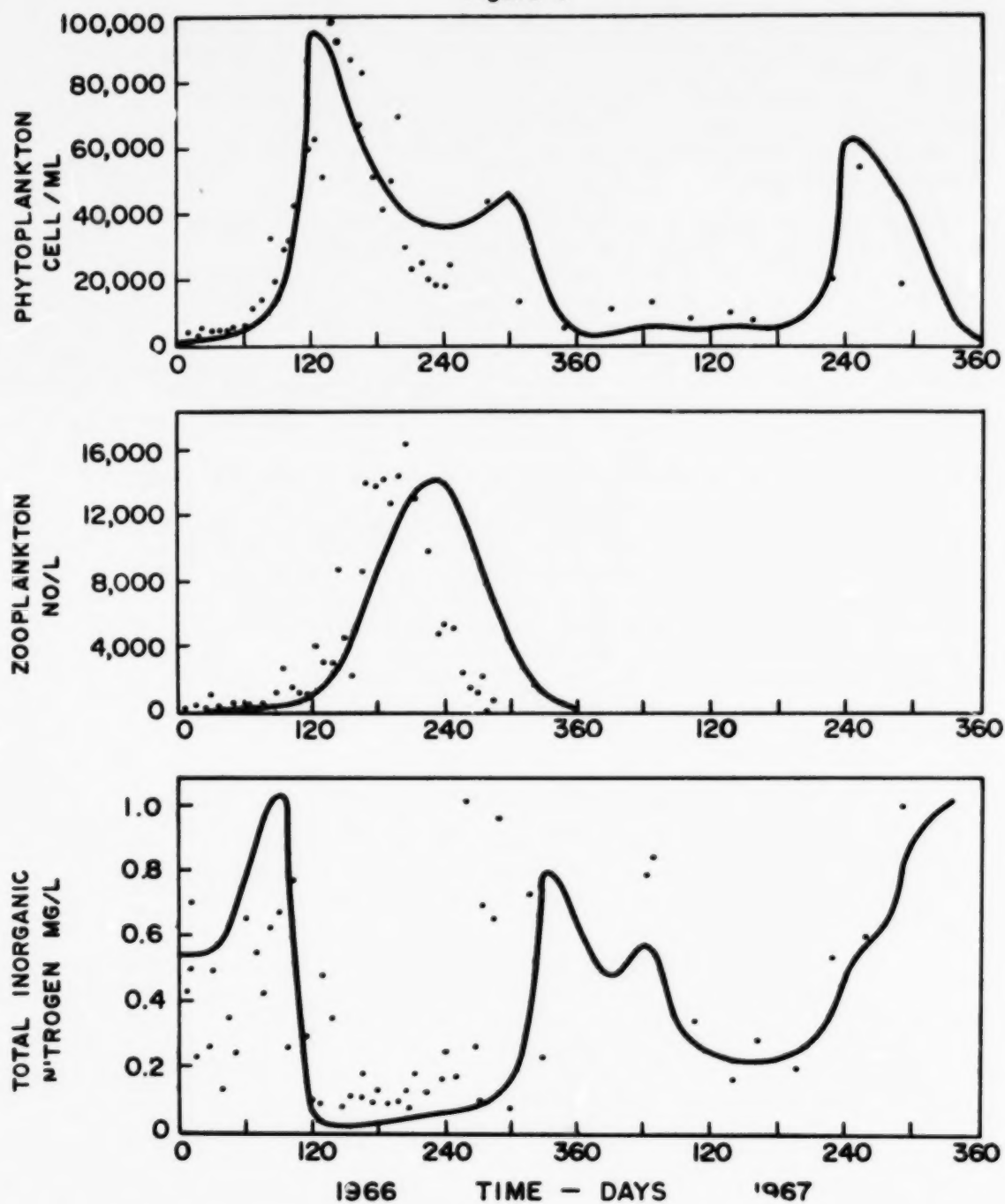
Figure 4 illustrates the degree of real data simulation afforded by the predictive model of DiToro, et. al. (198) for the San Joaquin River near Mossdale, California. Phytoplankton, zooplankton and total inorganic nitrogen simulations are shown. Inputs to the model included inorganic nitrogen (limiting nutrient), temperature, solar radiation and the advective flow.

Brezonik and Shannon (201) developed a Trophic State Indicator (TSI) which is computed from seven indicators: 1. primary production (PP), 2. Chlorophyll a (CHA), 3. total organic nitrogen (TON), 4. total phosphorus (TP), 5. Secchi Disc transparency (SD), 6. conductivity (COND), and 7. cation ratio ($CR = \frac{Na + K}{Mg + Ca}$) Table 2 taken from their publication presents the relationships derived for colored and clear water Florida Lakes.

By calculating regression equations between the TSI and land use factors, the equations on Table 3 were developed. The first equation considers TSI as linear function of land use patterns within the watershed plus immediate and remote cultural units. The second equation considers TSI as a function of land use patterns and total cultural units (Sum of remote, immediate and sewage treatment cultural units). Table 4 and figures 5 and 6 present the calculated critical phosphorus and nitrogen loading rates for Florida Lakes.

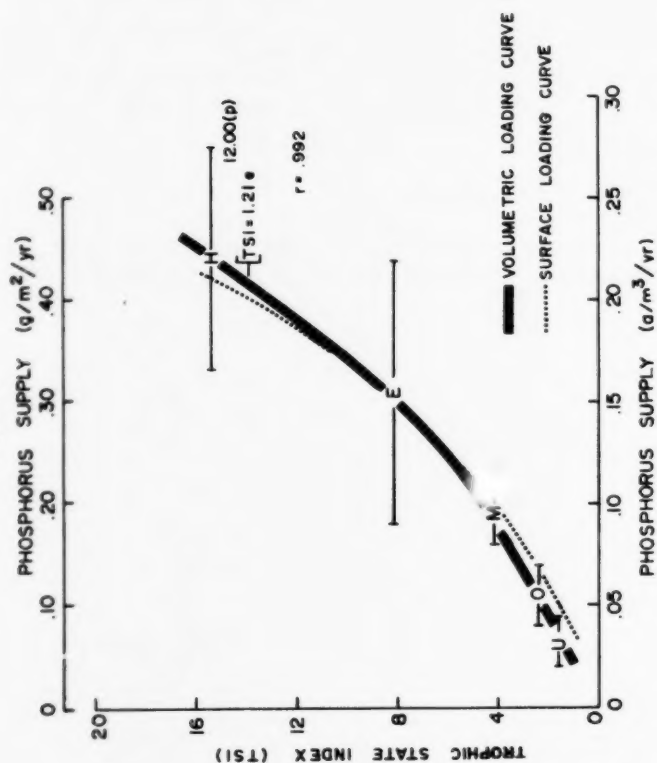
Although the results obtained by Brezonik and Shannon cannot be directly applied to lakes in other sections of the country without extensive verification, similar relationships may exist for other sets of lakes perhaps, regional location or morphological similarity. Such relationships could then be established by using similar methodology.

Figure 4



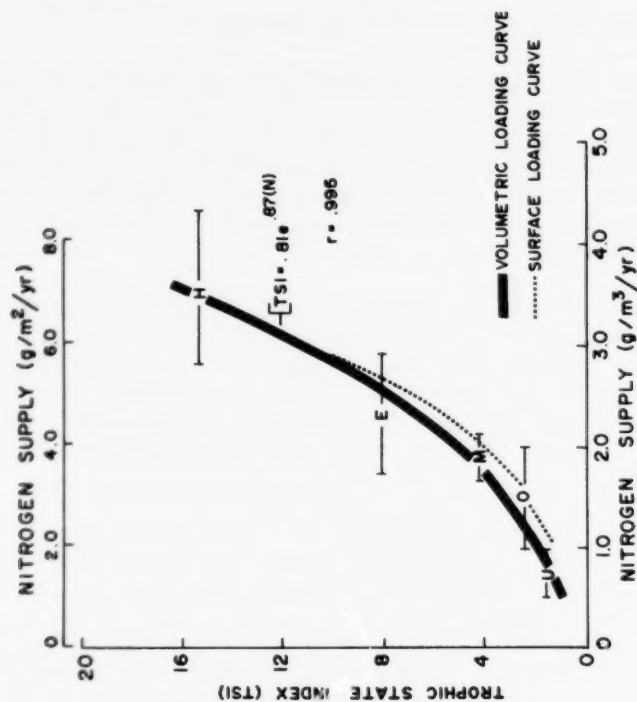
PHYTOPLANKTON, ZOOPLANKTON, AND TOTAL INORGANIC NITROGEN.
COMPARISON OF THEORETICAL CALCULATIONS AND OBSERVED DATA.
SAN JOAQUIN RIVER, MOSSDALE CALIFORNIA, 1966-1967 (198)

Figure 5



MEAN TSI VALUES FOR FIVE TROPHIC GROUPS vs. ANNUAL PHOSPHORUS LOADING IN g/m²-yr and g/m³-yr.
BRACKETS INDICATE RANGE FOR ONE STANDARD ERROR. SYMBOLS OF TROPHIC GROUPS ARE: ULTRAOLIGOTROPHIC (U), OLIGOTROPHIC (O), MESOTROPHIC (M), EUTROPHIC (E), HYPEREUTROPHIC (H) (201)

Figure 6



MEAN TSI VALUES FOR FIVE TROPHIC GROUPS vs. ANNUAL NITROGEN LOADING IN g/m²-yr and g/m³-yr.
SEE FIGURE 5 FOR EXPLANATION OF SYMBOLS (201)

Optimum or Cost-effective Solution

The models developed in previous sections in essence are "production functions." That is, for a given level of nutrient input, a certain level of phytoplankton biomass or trophic state level will be predicted. By testing the reduction of nutrient inputs in the model, a corresponding reduction in biomass for the prototype system is predicted.

Evaluation of all available and feasible alternatives by using technical, economic and political criteria, the various possible solutions can be formulated in monetary terms. Testing these available solutions with the corresponding natural system responses can ultimately lead to the most cost-effective solution of a problem.

As an example, assume that the phytoplankton biomass production function for a specific lake has been determined. The various wastewater treatment alternatives available to a city located on the lake might be advanced waste water treatment, disposal on nearby farmland by spray irrigation, or diversion around the lake of all waste discharges. Each of these alternatives have costs which can be evaluated. A model can be used to calculate which individual or combination of alternatives can meet the lake requirements in the most cost-effective manner. A solution would then be recommended. Suitable nutrient removal techniques and estimated costs have been described by Rohlich and Uttormark (202) and Swanson (203).

Post Project Evaluation

Projects involving nutrient removals as part of the waste treatment system are relatively new. The actual

Table 3

Stepwise Regression Analysis of Trophic State Index vs.
Eutrophication Factors Expressed Per Unit Lake Volume

(1) Regression Equation:

$$\begin{aligned} \text{TSI} = & 14.95(\text{HFA}) + .64(\text{FOR}) + 2.72(\text{ICU}) + 1.59(\text{URB}) \\ & \quad 59.6 \quad \quad 73.9 \quad \quad 80.0 \quad \quad 81.2 \\ & - .35(\text{UCA}) + .06(\text{RCU}) - .02(\text{PA}) \\ & \quad 81.5 \quad \quad 81.5 \quad \quad 81.5 \end{aligned}$$

F Ratio = 28.98***

Multiple Correlation Coefficient (r) = .903

Percent of total variation explained by the
regression equation = 81.5%

(2) Regression Equation:

$$\begin{aligned} \text{TSI} = & 14.49(\text{HFA}) + .61(\text{FOR}) + 2.23(\text{URB}) + .53(\text{TCU}) \\ & \quad 59.6 \quad \quad 73.9 \quad \quad 79.4 \quad \quad 80.0 \\ & + .31(\text{UCA}) - .01(\text{PA}) \\ & \quad 80.3 \quad \quad 80.3 \end{aligned}$$

F Ratio = 31.91***

Multiple correlation coefficient (r) = .896

Percent of total variation explained by the
regression equation = 80.3%

Key to Eutrophication Factor Symbols:

HFA = Heavily fertilized cropland (m^2/m^3)
FOR = Forested area (m^2/m^3)
ICU = Immediate cultural units ($\#/\text{m}^3 \times 10^4$)
URB = Urban area (m^2/m^3)
UCA = Unproductive waste cleared area (m^2/m^3)
RCU = Remote cultural units ($\#/\text{m}^3 \times 10^4$)
PA = Pastured area (m^2/m^3)
TCU = Total cultural units ($\#/\text{m}^3 \times 10^4$)

***Denotes significant F value at the 99% confidence level.

Values below symbols in regression equation indicate
cumulative percent of total variance explained by
independent variables up to that point.

changes which occur in bodies of water following institution of such treatment should be thoroughly documented on a year by year basis. Such accumulated information will provide knowledge on actual system responses versus those predicted. The validity of assumptions, bio-kinetic formulations, species dominance changes and bottom mud-water interactions are types of data needing verification. Such information is required before nutrient removal waste treatment programs reach maximum expenditure levels so as to avoid many treatment mistakes and still provide for corrective action.

In addition to receiving system responses, actual operating experience with new treatment systems is necessary to obtain actual removals, consistency of operation and costs.

Another problem which must be answered is model extrapolation especially with mathematical models that must be extended far outside of the data ranges used in their development. Such situations can occur with models developed for grossly over-fertilized lakes and are used to extrapolate conditions for achieving oligotrophy. Another facet of this problem is the calculation of lake response following the removal of only one nutrient to extremely low levels. Current models might not identify actual effects even approximately and severely unbalanced biological systems might develop in the prototype. It is conceivable that extremely effective removal of phosphorus without complementary carbon removal might lead to the development of detritus accumulations because some link in the food chain may be limiting. Such facets of mathematical modeling of the eutrophication problem have not received adequate attention.

Table 4

Critical Loading Rates for Nitrogen and Phosphorus

Reference	Loading Rate Units	Permissible Loading (up to)		Dangerous Loading (in excess of)	
		N	P	N	P
201	Volumetric (g/m ³ -yr)	.86	.12	1.51	.22
201	Areal (g/m ² -yr)	2.0	.28	3.4	.49
200	Areal	1.0	.07	2.0	.13

^aFor lakes with mean depths of 5 m or less.

From: Brezonik and Shannon (201)

RECOMMENDATIONS

An approach to understanding the role of microorganisms in the environment can be developed as separate categories. All of these areas are interrelated, but each can be studied independently. Results from each of these studies can be collated and used to evaluate problems, develop better waste disposal methods and water management, allow for the establishment of meaningful criteria, and enforce existing regulations.

I. NUTRIENT CYCLING

The following areas are in need of consideration and investigation with respect to nutrient cycles:

- (a) Kinetics and dynamics of organic and inorganic nutrient turnover in microbial populations: The approach necessitates dealing not only with the effects of single pollutants, but also with the possible synergistic effects of combinations of pollutants in relation to physical and biological environmental conditions.
- (b) Identification of microorganisms which contribute characteristic tastes and odors in waters: For example, Actinomyces species are highly suspect as causative agents for many noxious odors and tastes. Other microbes can deposit metallic iron and other elements such as sulfur. Certain bacteria produce slimes or taste producing substances (83). The relationship between selective growth of these

odor producing organisms and eutrophication requires investigation. Identification of indicator organisms of specific classes of pollutants is a possibility. Clostridium perfringens, a causative agent for gas gangrene, is an anaerobic spore former found as a normal inhabitant in the digestive tract of man and other animals. However, little information is available on the role this organism might play as an indicator of fecal pollution (85). This is especially important since, as a spore former, samples can be stored for long periods of time without loss of viability. The medical significance of this organism in relation to gastroenteritis is not clearly understood and requires clarification.

- (c) Defining food chain relationships beginning with the microorganisms is particularly important, especially when considering possible modification of toxic pollutants and sequestering of metals that in high concentrations, become toxic for humans and other animals. Cadmium, lead and mercury, as well as certain pesticides, of which DDT is an example, are concentrated by microorganisms and are further magnified in the food chain.
- (d) The effect of environmental, physical, and chemical limitations on microbial growth; e.g. carbon dioxide, oxygen, pH, light and temperature, is not known with respect to the overall aquatic ecosystem. Alterations of any of these parameters through excessive eutrophication or pollution needs additional research.

- (e) Nitrogen fixation by microorganisms in various fresh water, and estuarine and ocean environments should continue to be considered. Investigations into the quantity of nitrogen fixed as related to the total productivity are required.
- (f) The sulfur cycle is an area that has not been fully explored and a delineation of the cycle as a primary energy cycle in certain estuaries and lakes requires investigation.

III.

ANAEROBIC MICROORGANISMS

A consideration of the metabolic activities as related to eutrophication processes, self-purification capacity of water, and potential problems related to septic conditions of strict anaerobic bacteria and other microorganisms and benthic organisms needs to be initiated. Little is known about the effect on either the viability or selection various nutrients or pollutants have on the anaerobic flora.

IV.

MICROBIAL PHOTOSYNTHESIS

An evaluation of the role of photosynthetic microorganisms to eutrophication and to oxygen requirements is required. This includes not only the blue-green algae, but also the purple sulfur bacteria as well as other species capable of photosynthesis.

V.

INTERSPECIES INTERACTION

An examination of the interplay between microorganisms as related to and determined by the water quality is required. For example, if bacteria of one species is given a growth advantage over the rest of the bacterial flora can the whole microbial relationship be altered with a consequent impact on the food chain.

VI.

Engineering Aspects

a. Further study to determine the limits of model extrapolation and to obtain cost-effective solutions of accelerated eutrophication problems is required before large sums of money are spent on remedial measures based on such extrapolations.

b. Surveillance of systems where nutrient removal is instituted to verify the validity of assumptions, bio-kinetic formulations and predicted water quality changes.

VII.

METHODS

The methods would involve:

- (a) Separation and enumeration of microbial species in natural populations.
- (b) Estimation of activity rates in situ with respect to synthesis, degradation and cycling of organic and inorganic materials, this would include a study of the following general areas:
 - 1. anaerobic environments
 - 2. aerobic environments

3. biological vs. abiological oxidation

- (c) Identifying microbial populations in specific ecological niches. For example, do specific microorganisms associate with specific higher life forms and, if so, how does the elimination of these bacterial species affect these higher life forms, i.e., protozoa, fungi, rotifers, and plants?
- (d) Development of techniques required for the identification on higher microorganism, i.e., rotifers, algae, protozoa

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